

Effect of Heat Treatments on Stability of Penicillin in Waste *Penicillium Chrysogenum*

Pu Wang¹, Huiling Liu^{*2}, Huan Fu³, Xiuwen Cheng⁴, Bing Wang⁵, Qinghua Cheng⁶, Jian Zhang⁷

^{*1, 2, 3, 4, 5, 6, 7}Department of Environmental Science and Engineering, Harbin Institute of Technology
Harbin, China

^{*2}hlliu2002@126.com

Abstract

Penicillin G (PENG), one of the most widely used bacteriostatic antibiotics in human and veterinary medicine, may cause biological drug resistance and environmental toxicity. In this study, the degradation efficiency of PENG has been investigated by heat treatment. A kinetic study was performed under different temperature conditions (60°C, 70°C, 80°C, 90°C and 100°C). The first-order rate constants for the direct reaction of PENG with heat treatment were measured to be 0.008 to 0.027 under the temperature conditions tested. The rate constants were greater at higher temperature. These results have suggested that higher temperature conditions and short term are needed for the removal of PENG by heat treatment in order to mitigate the residual toxicity that can remain in waste *penicillium chrysogenum* manufactured during the penicillin pharmaceutical process.

Keywords

Penicillin G; Waste *Penicillium Chrysogenum*; Temperature; First-Order Rate

Introduction

Penicillin has been widely used as antimicrobial drugs for more than 80 years and considered as one of important groups of antibiotics. The first antibiotic discovered, penicillin G, appeared in the market just after the Second World War. Since then as the only natural penicillin available in the market, it is an antibiotic produced by *Penicillium chrysogenum*, which includes in the structure a lactamic ring and a carboxylic group. Due to its large activity, this antibiotic is often used against both aerobic and anaerobic bacteria, being an important drug used in prevention and treatment of bacteria infections.

However, during the production of penicillin, a mass of waste *penicillium chrysogenum* is generated. Recently, it was found that the *penicillium chrysogenum* contains rich high-quality protein, fat, cellulose, a group of enzymes

and other components, especially variety of essential amino acids is available [Cheng & Zhang (2003), Yao & Moellering, Jr. (1999)]. In addition, the nutritional value of the *penicillium chrysogenum* is equal to soybean meal, which is suitable as animal feed. Unfortunately, doubts of its suitability as a feedstock have been raised because of the small amount of antibiotics and the degradation products remaining in the bacterial residue. The residue is toxic and dangerous for human health, and may cause allergic reactions and antimicrobial resistance [Guillemot et al. (1998), Wilke et al. (2005)]. So, penicillin bacterial residue is one of the hazardous wastes now (National Catalogue of Hazardous Wastes, China) (The People's Republic of China Ministry of Environmental Protection and National Development and Reform Commission, 2008). Therefore, penicillin bacterial residue should be carefully managed due to the hazardous waste.

In the previous research, there is few paper focused on the characteristics and stability treatment of waste *penicillium chrysogenum*. The heavy metal, polycyclic aromatic hydrocarbons and other basic constituent content were reported by Bin Guo et al. [Guo et al. (2012)]. In 2007, Wang, T.Q. et al. [Wang et al. (2007)] reported a method to extract ergosterol (1-3)- α -D-glucan and chitosan from *Penicillium chrysogenum*, however, it is high-cost but low economic benefits.

Therefore, the aim of this paper was to investigate the effect of the temperatures and times of heat treatments on the residue of PENG in waste *Penicillium chrysogenum* using HPLC method. Meanwhile, the degradation kinetic model was investigated as well.

Materials and Methods

Chemicals and Reagents

Penicillin G ($\geq 99.0\%$) was purchased from Sigma

Aldrich (Product number P-7794; Sigma). Acetonitrile and formic acid (HPLC grade) were obtained from J&K Technology Company (Harbin, china). The other reagents used were of analytical grade. Milli-Q quality water (Millipore, Bedford, MA) was used during the whole analysis.

Stock standard solutions ($2000 \text{ mg}\cdot\text{L}^{-1}$) prepared with acetonitrile: water (1:1, V/V), was stored in dark below 4°C . Working standard solutions was diluted by acetonitrile: water (1:1, V/V) freshly before analysis.

Penicillium Chrysogenum Samples and Heat Treatment

The waste *penicillium chrysogenum* was collected from the pharmaceutical company in China. The penicillium chrysogenum were placed in polyethylene bags and stored at -20°C . Samples prior to treatment were then divided into aliquots to study the effect of different temperatures (60, 70, 80, 90, and 100°C). The penicillin G residue was measured after heat treatment, the time interval was 0 min, 30 min, 60 min, 120 min, 180 min and 240 min, respectively.

Extraction Procedure

Waste *penicillium chrysogenum* samples were homogenized by mortar. Then, the homogenized sample was added into a centrifuge tube and mixed with mixture of acetonitrile and formic acid (3:1, V/V). After that mixture was vortex for 30 s and then extracted through ultrasonic for 30.0 min. Subsequently, the mixture was centrifuged for 5.0 min, and then the supernatants were transferred into another centrifuge tube containing n-hexane. Then it was centrifuged for 5.0 min. 1.0 mL upper layer was collected and filtered through a $0.45 \mu\text{m}$ organic filter, ready for injection into HPLC.

HPLC-DAD Analysis

A Waters high performance liquid chromatography connected to diode array detector was employed. An instrument online workstation (Waters) was utilized to control the system and for the acquisition and analysis of the data. A Waters- C_{18} column ($4.6 \times 150 \text{ mm i.d.}$, $5.0 \mu\text{m}$) was used for separations and column temperature was kept at 30°C . The injection loop volume was $10.0 \mu\text{L}$. The mobile phase was a mixture of acetonitrile: 0.3% (v/v) of formic acid in water system (40:60; v/v). The flow rate was $1.0 \text{ mL}\cdot\text{min}^{-1}$. The DAD monitoring wavelength was chosen to be 215 nm .

Penicillin G was identified according to the retention time and. Quantification was based on peak area ratio

of the target analyses divided by the external standard.

Statistical Analysis

First-order dissipation kinetics $\text{Ln}y = \text{Ln}A_1 - kt$, where y = concentration after time t , A_1 = apparent initial concentration, and k = rate constant) were used to interpret the penicillin G extractable residues data. Respective equation correlation coefficients and the fitting levels were calculated. From the data obtained, the $T_{1/2}$ (half-dissipation time) was calculated.

Results and Discussions

Figure 1 shows a typical HPLC chromatogram of the Penicillin G standard solution and the waste *penicillium chrysogenum* sample. Quantitative analysis was conducted using an external standard. Good linearity of the DAD response was found for penicillin G at concentrations within the test intervals, with linear range from 0.5 to $2000.0 \mu\text{g}\cdot\text{mL}^{-1}$ with a linear regression coefficient (R^2) 0.9998 .

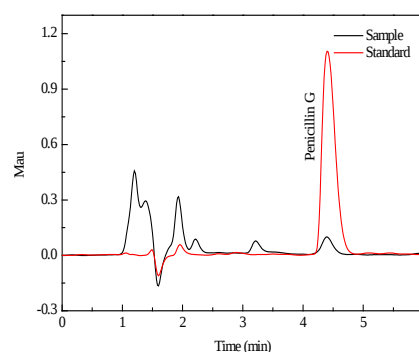


FIGURE 1 THE HPLC CHROMATOGRAM OF PENICILLIN G STANDARD SOLUTION AND THE WASTE *PENICILLIUM CHRYSOGENUM* SAMPLE.

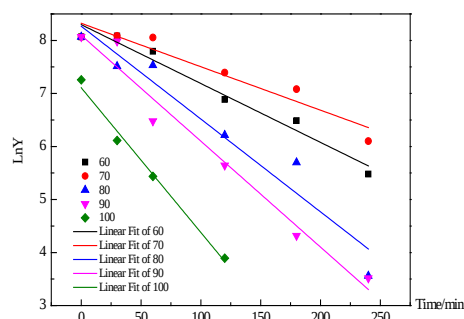


FIGURE 2 DECREASED CONCENTRATION OF PENICILLIN G IN WASTE *PENICILLIUM CHRYSOGENUM* AT DIFFERENT HEATING TIMES AND TEMPERATURES

The degradation of the penicillin G in waste *penicillium chrysogenum* for heat treatment at different temperature is reported in Figure 2. The values of Intercept ($\text{Ln}A_1$), Slope (k), fitting level (R^2), half-dissipation time ($T_{1/2}$) and F-value are labelled in Table 1. In all cases, the quadratic regression coefficients (R_2) were high ranging from 0.904 to 0.985, indicating that the kinetic model was valid to study the thermal degradation of penicillin G in waste *penicillium chrysogenum*. It is also noteworthy in Table 1 that the effect of heat treatment time on the thermal degradation of penicillin G is highly significant in all cases, and the degradation of penicillin G has an obvious significant positive correlation with temperature except 70°C. Table 1 shows the half-lives of Penicillin G in waste *penicillium chrysogenum* estimated with the first-order kinetic equations for the 5 temperatures assessed. Thus, the order of degradation rate of Penicillin G is 100°C>90°C>80°C>60°C>70°C, in contrast the half-lives is from 9.73 to 116.05 min, respectively. Greater half-lives (Table 1) were found for penicillin G at lower temperature, indicating that the greater thermal instability of penicillin G, with shorter half-lives at 100°C, is only 9.73 min.

TABLE 1 PARAMETERS OF THE FIRST-ORDER DEGRADATION KINETIC MODEL OF PENICILLIN G IN WASTE PENICILLIUM CHRYSOGENUM AT DIFFERENT TEMPERATURES

Temperature (°C)	Intercept ($\text{Ln}A_1$)	Slope (k)	F-value	R^2	$T_{1/2}(\text{min})$
60	8.29	-0.011	132.08	0.963	83.35
70	8.32	-0.008	47.98	0.904	116.05
80	8.27	-0.018	66.36	0.929	51.17
90	8.10	-0.020	137.92	0.965	36.29
100	7.11	-0.027	201.66	0.985	9.73

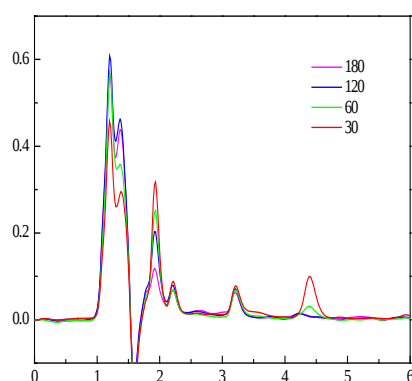


FIGURE 3 THE THERMAL DEGRADATION OF PENICILLIN G IN WASTE PENICILLIUM CHRYSOGENUM AT DIFFERENT INTERNAL TIME WITH 100°C HEAT TREATMENT

Figure 3 illustrates the thermal degradation of penicillin G in waste *penicillium chrysogenum*. Greater

thermal degradation of penicillin G can be observed when heating time was prolonged and temperature increased. The property of β -lactams could be explained by the fusion of the β -lactam ring with the thiazolidine ring, which leads to non-planarity of molecules, resulting in wide-angled and torsional rotation [Cohen (1983), Deshpande et al. (2004)]. This labile molecular structure is characterized by high susceptibility to various nucleophiles, acid and base reagents, metal ions, oxidizing agents, and even solvents like water [Guillemot et al. (1998)]. Therefore, the degradation of penicillin G observed in this study could be partly accounted for by the high water content of waste *penicillium chrysogenum*. (79%-86%).

Conclusions

A first-order kinetic model was used to explain the thermal degradation of penicillin G residues in *penicillium chrysogenum*. The use of predictive models helps in the estimation of thermal degradation of penicillin G for the different processes applied in recycle process of waste *penicillium chrysogenum*. The degradation half-lives of penicillin G decrease with increasing temperatures. Therefore, higher temperature, shorter time are more effective for heat treatment for waste *penicillium chrysogenum*. High temperature-short time treatment may provide a possible treatment to solve the antibiotics residue problem in recycle resource of waste *Penicillium chrysogenum*.

ACKNOWLEDGMENTS

This work was supported by the funds for National environmental charity technology project (201209024), Creative Research Groups of China (Grant No. 51121062) and State Key Laboratory of Urban Water Resources and Environment (2010DX03).

REFERENCES

- Cheng, J.H., and Zhang, W.L. 2003. Process design for bacterial antibiotic residue. *Pharmaceut. Eng. Design* 24:31–34.
- Cohen, N. C. 1983. β -Lactam antibiotics: Geometrical requirements for antibacterial activities. *Med. Chem.* 26:259–264.
- Deshpande, A. D., Baheti, K. G., and Chatterjee, N. R. 2004. Degradation of β -lactam antibiotics. *Curr. Sci.* 87:1684–1694.

- Guillemot, D., Carbon, C., Balkau, B., Geslin, P., Lecoœur, H., Vauzelle-Kervroedan, F., Bouvenot, G., and Eschwege, E. 1998. Low dosage and long treatment duration of β -lactam. Risk factors for carriage of penicillin-resistant *Streptococcus pneumoniae*. *JAMA* 279:365–370.
- Guo, Bin, Gong, Lipeng, Duan, Erhong, Liu, Renping, Ren, Ailing, Han, Jing & Zhao, Wenxia. Characteristics of penicillin bacterial residue, *Journal of the Air & Waste Management Association*, 62(4):485–488, 2012.
- Wang, T.Q., Li, H.X., Wang, M.Y., and Tan, T.W. 2007. Integrative extraction of ergosterol (1-3)- α -D-glucan and chitosan from *Penicillium chrysogenum* mycelia. *Chin. J. Chem. Eng.* 15:725–729.
- Wilke, M., Lovering, A. L., and Strynadka, N. 2005. β -Lactam antibiotic resistance: A current structural perspective. *Curr. Opin. Microbiol.* 8:525–533.
- Yao, J.D.C., Moellering, R.C., Jr., in: P.R. Murray, E.J. Baron, M.A. Tenover, F.C. Tenover, R.H. Tenover (Eds.), *Manual of Clinical Microbiology*, American Society for Microbiology, 1999, pp. 1474–1475.